Effectiveness of AQUI-S20E on Marine Fish:
Sedation to Handleable

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DEPARTMENT OF THE INTERIOR

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Fish Sedatives

- **Agents that, with increasing concentration and duration…**
  - Calm an animal and then cause successive loss of mobility, equilibrium, consciousness, and reflex action
- **Routinely used in fisheries to minimize stress to fish when…**
  - Collecting tissue samples or morphometric data
  - Surgically implanting tags
  - Sorting (field and hatchery); staging; transportation
- **Fish are sedated to…**
  - Minimize mechanical damage
    - Epithelium/internal tissues
  - Reduce physiological consequences of handling
    - Generalized stress response
  - Address animal welfare
    - Pain or nociception

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Sedative Options

- **MS-222 - approved by FDA**
  - **21-d withdrawal period**
- **Clove Oil**
  - **Not approved by FDA for use on fish**
- **CO2 (gas/alka seltzer/sodium bicarbonate)**
  - **Not approved by FDA; low regulatory priority; not all fish respond well**
- **AQUI-S 20E (10% eugenol)**
  - **Potential immediate-release sedative**

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Immediate-release Sedatives

- **It's impractical to hold fish after sedation**
  - Potential to severely constrain field activities
  - Use of clove oil or MS-222 off label violates parts of the FFDCA
- **AQUI-S 20E is the best option**
  - Currently available under the USFWS INAD exemption authorization
    - Immediate release for field use on freshwater finfish; 3-d w/d for all other uses
  - Initial approval
    - Sedation of freshwater salmonids to handleable – 2017
  - Effective
    - Sedate fish within 5 min
    - Preferably within 1 – 3 min
    - Lose equilibrium & ability to swim
  - Safe to fish
    - 1× dose: 3 – 4 min ≥ Time to sedation
    - 1.5× dose: 2 – 3 min ≥ Time to sedation

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AQUI-S20 is effective
Freshwater fish

Target Dose	Sedation	Recovery
25 mg/L (4 salmonids/14°C)	2.0 min (range, 1.3 – 3.2)	6.2 min (range, 3.6 – 10.8)
40 mg/L (4 coolwater species/18°C)	2.1 min (range, 1.4 – 3.1)	8.0 min (range, 5.9 – 10.2)
60 mg/L (4 warmwater species/24°C)	1.4 min (range, 0.9 – 1.7)	8.0 min (range, 5.2 – 12.9)

Effectiveness Technical Section complete (May 16, 2013)

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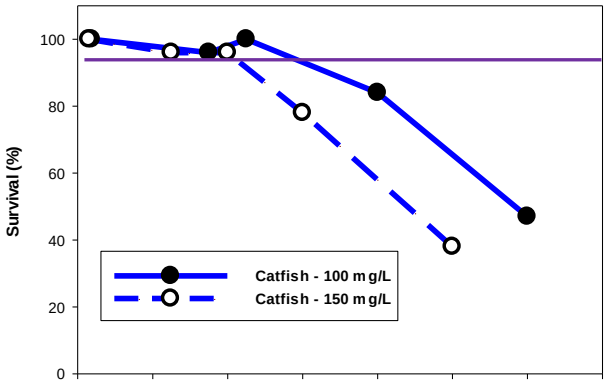
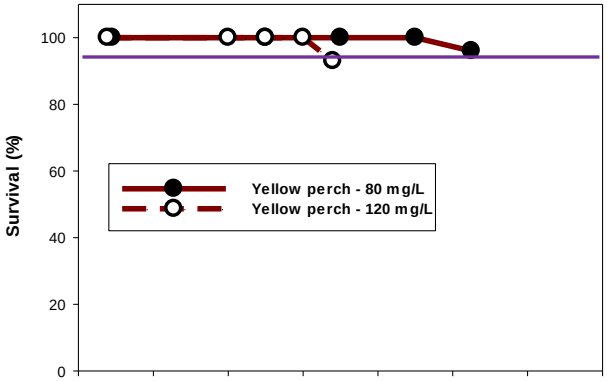
Catfish survival (%)

100 mg/L eugenol – to 4.5 min
(Sedation – 0.25 min)
150 mg/L eugenol – to 4.0 min
(Sedation - 0.25 min)



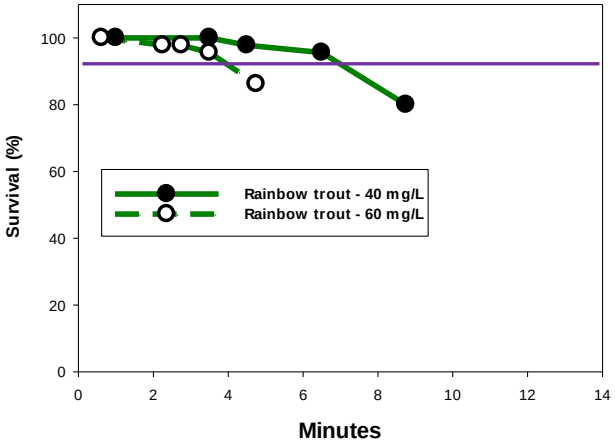
Yellow perch survival (%)

80 mg/L eugenol – to at least 10.5 min
(Sedation - 1 min)
120 mg/L eugenol – to 6.0 min
(Sedation – 0.75 min)



Rainbow trout survival (%)

40 mg/L eugenol – to 6.5 min
(Sedation – 1 min)
60 mg/L eugenol – to 3.5 min
(Sedation – 0.5 min)



Technical section complete 5/29/2014

Marine fish sedation

- **Demonstrate that AQUI-S20E effectively sedates marine fish to handleable in seawater**
  - **CVM concurred protocol**
  - **Virtually identical to the protocol used for freshwater fish**
- **Generate data to complete effectiveness technical section**
  - **Test 6-8 different fish species**
- **Do marine fish respond similarly to freshwater fish?**
  - **Test fish at:**
    - **Similar temperatures, doses**
  - **Compare times to sedation/recovery**
    - **Freshwater fish data**

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Fish species tested

Done

Warmwater

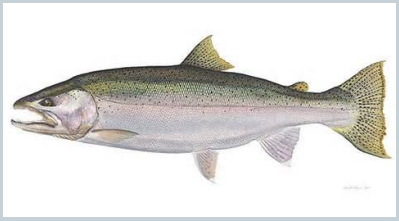
Black Sea Bass

Cobia

Pompano

Coldwater

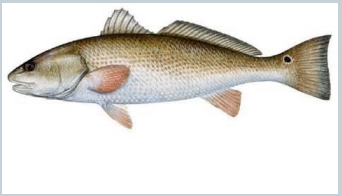
Sablefish



Steelhead Trout

To be tested (possibly)

Warmwater



Red Drum

Coldwater

Atlantic Salmon

White Sea Bass

Yellowtail Amberjack

4 MUMS grant applications approved for funding

Testing

Species	Size (cm)	Dose		Water temp (°C)
		Eugenol	MS222	
Pompano	9.9 ± 1.1	30	120	24.5
Pompano	15.7 ± 1.3	30	120	24.2
Cobia	16.0 ± 1.2	30	120	25.1
Black Seabass	19.6 ± 2.1	30	120	24.7
Steelhead	26.7 ± 1.7	25	80	12.5
Sablefish	25.4 ± 3.8	60	180	11.1

30 fish tested separately with AQUI-S20E or MS222

Sedated in static bath, exchanged after every fish

Recovered in flow through (pompano/cobia) or static (all others)

Assessed behavior during sedation and recovery; water temp and DO - every fish

Eugenol dose verification - every sedation bucket

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Time to sedation/recovery

Species	Time to sedation		Time to recovery	
	Eugenol	MS222	Eugenol	MS222
Pompano (S)	0.9	1.1	4.3	3.1
Pompano (L)	1.1	1.3	5.0	3.6
Cobia	2.1	1.4	6.7	3.6
Black Seabass	0.8	1.0	5.7	2.9
Sablefish	1.6	1.1	7.9	7.0
Steelhead	1.7	1.3	4.0	3.9

Marine warmwater fish treated with 30 mg/L eugenol and 120 mg/L MS222
- freshwater with 60 mg/L eugenol and 150 mg/L MS222
Steelhead trout in seawater time to sedation comparable to freshwater salmonid times
- all tested at 25 mg/L eugenol
Sablefish (deep water fish) response similar to sturgeon
- difficult to assess
All fish recovered

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Status and next moves

- **Final Study Reports submitted to CVM**
  - **CVM response 5/16/2016**
    - **Acceptable as substantial evidence of effectiveness**
      - **Technical section remains incomplete**
    - **May consider the TAS study on RBT adequate for demonstrating safety to seawater salmonids**
- **DRIBS 'published'**
  - **#48 and #49 on AADAP website**
- **Submit MUMS grants to fund remaining studies**
  - **Yellowtail Amberjack and White Seabass**
    - **Hubbs SeaWorld and Research Institute**
  - **Atlantic Salmon**
    - **e.g., University of Maine**
  - **Red Drum**
    - **e.g., FAU Harbor Branch Oceanographic Institute**

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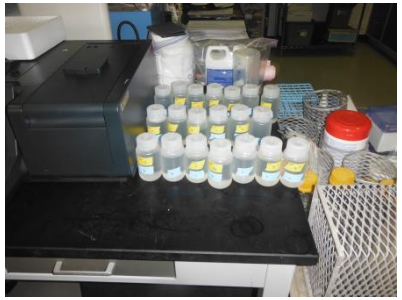

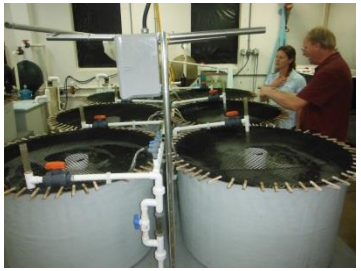

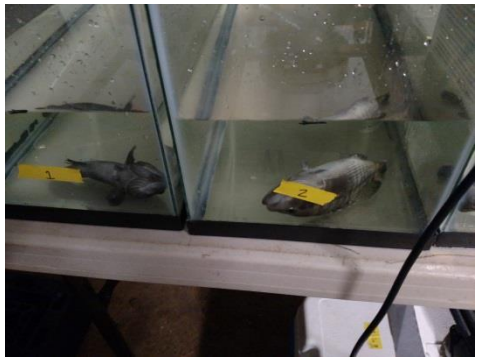

Questions? Questions? Questions?